**1119-Pos Board B11**

**Effects of Lipids on the Structure and Function of GLIC and ELIC**

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The prokaryotic pentameric ligand-gated ion channels (pLGICs), GLIC and ELIC, are excellent models for probing the mechanisms of pLGIC function. We are interested in the mechanisms by which lipids act as allosteric modulators of the prototypic pLGIC, the nicotinic acetylcholine receptor (nAChR) from *Torpedo*. In mixed phosphatidylcholine (PC) membranes such as those formed from soybean asolectin or those containing neutral and/or anionic lipids, the nAChR adopts an activatable resting conformation. In PC membranes lacking neutral and anionic lipids, the nAChR is stabilized in an uncoupled conformation that binds agonist with resting-state like affinity, but does not undergo conformational transitions. To test whether GLIC and ELIC exhibit similar lipid dependencies, we developed a protocol for reconstituting both GLIC and ELIC into model membranes composed of either soybean asolectin or PC. Both reconstituted pLGICs exhibit secondary structures similar to that of the nAChR, but both have substantially enhanced thermal stabilities, a likely requirement for the formation of diffraction quality crystals. Whereas incorporation of the nAChR into PC membranes leads to an increase in peptide hydrogen exchange relative to the nAChR in asolectin, this was not observed with GLIC and ELIC. In addition, the functional properties of both were explored by two-voltage electrode clamp electrophysiology. Our results suggest that although both GLIC and ELIC exhibit a functional sensitivity to their lipid environments, these sensitivities differ substantially from those of the *Torpedo* nAChR. These functional differences will be discussed in light of the current structures of these three pLGICs.

**1120-Pos Board B12**

**The Release Pathway of Copper Transporting Ptype ATPases**

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Cellular levels of heavy metals are carefully regulated by the PIB class of P-type ATPases in all kingdoms of life and mutations of the human members ATP7A and ATP7B are the cause of the severe Menkes' and Wilson's diseases. Recently, a crystal structure of a homologous $Cu^+$ ATPase from Legionella pneumophila (LpCopA), trapped in a transition state of dephosphorylation (E2Pi), suggested that copper extrusion employs an intramembranous exit site, but the release pathway remained elusive and the transmembrane (TM) domain was inferred to be occluded. However, by molecular dynamics (MD) simulations, we find that extracellular bulk water solvates the proposed exit and high-affinity ion-binding sites deep within the membrane. This view found further support by a 2.8 resolution LpCopA crystal structure trapped in the E2P state (associated with extracellular exchange in well-known PII-type ATPases such the sarcoplasmic reticulum $Ca^{2+}$-ATPase, SERCA) showing a similar structure of the TM-domain, and delineating the same pathway by crystal water positions. We conclude that the E2P and E2Pi states are equally open, indicating that $Cu^+$ ATPases couple the conformational changes associated with ion extrusion differently to dephosphorylation as compared to SERCA; in accordance with structural differences. The observed copper extrusion conduit was further validated by mutational studies and shown to involve the PIB-specific MA segment, which is absent in e.g. $Co2^+$ ATPases and thus different unloading schemes may apply within PIB-ATPases. The pathway further explains why Menkes' and Wilson's mutations at the extracellular side impair protein function and constitutes a favorable site for novel inhibitors targeting pathogens from the extracellular environment.

**1121-Pos Board B13**

**The Disordered N-Terminus of the Plant Antenna Protein CP29 Studied by Electron Paramagnetic Resonance - Is this 100-Residue Stretch Unstructured?**

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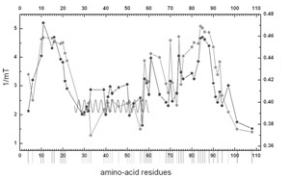
The N-terminal domain of the photosynthetic light-harvesting protein CP29, a membrane-antenna protein of PSII, is considered relevant for light-adaptation of the organism,[1] but was not resolved in the recent crystal structure of CP29.[2] We investigate the 100 amino-acids missing in that structure by site-directed spin-label EPR.[3] Mobility of the spin labels reveals that the N-terminus of CP29 is relatively structured and consists of at least five regions differing in their dynamics (Fig.). Remarkable are relatively immobilized regions, comprising the first six residues of the N-terminus and a stretch of residues from 30 to 60 with a potential α-helical region, possible attachment points to the protein surface. Distances from Double Electron-Electron Resonance (DEER or PELDOR) show defined but multiple distances, suggesting multiple conformations.




We propose that the N-terminus is flexible, but not disordered, which may be functionally important.

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**1122-Pos Board B14**

**Interactions between Anti-HIV Antibodies and their Lipid-Embedded Epitopes Defined by EPR Spectroscopy**

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A vaccine capable of stimulating protective anti-viral antibodies is needed to curtail the global AIDS epidemic. The membrane proximal ectodomain region (MPER) of the HIV envelope protein gp41 is the target of human neutralizing antibodies 4E10, 2F5, Z13e1 and 10E8. How these antibodies bind to their membrane-immersed epitopes and mediate anti-viral activity are unclear. Here, electron paramagnetic resonance (EPR) techniques were used to define the manner in which these antibodies recognize the L-shaped helix-hinge-helix MPER segment. Both 4E10 and 2F5 induce large conformational changes in the MPER relative to the membrane, and extracts buried residues from the lipids. The interaction is a stepwise and dynamic rearrangement through an apparent scoop-like movement of the antibodies' long and unique CDRH3 segments. Mutations of the CDRH3 segments reduced the ability of the antibodies to extract MPER peptides from membranes. These findings and others currently under investigation have significant implications for structure-aided vaccine design.

**1123-Pos Board B15**

**Probing the Conformation and Dynamics of Influenza a M2 Protein using Site-Directed Spin-Label EPR Spectroscopy**

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M2 is a membrane protein critical to the life cycle of influenza A. We have capitalized on the expanding body of high-resolution structural data available for the 97 amino acid M2 protein to design and interpret site-directed spin labeling electron paramagnetic resonance spectroscopy (SDSL-EPR) experiments on the conformation and dynamics of the homotetrameric M2 protein embedded in lipid bilayers. We have obtained data for three different M2 constructs (M2TM 22-46, M2TMC 23-60 and full length M2 protein) spin-labeled at multiple sites within the transmembrane and C-terminal domains. CW and pulsed EPR spectra show evidence that M2 adopts multiple conformational states in bilayers, and that cholesterol content dictates the relative populations of the states.

**1124-Pos Board B16**

**Structure-Function Studies of Mtb Membrane Protein CrgA in Lipid Bilayer**

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Tuberculosis is a deadly disease with a death toll of 1.5 million people every year and very recently an outbreak in Jacksonville, FL was devastating. All frontline antibiotics are failed for the multidrug resistant (MDR) bacilli and the key to disease control is to inhibit bacterial cell division and stabilize the bacilli in its dormant stage. Here we present the structure function studies of

a novel drug target membrane protein CrgA in M. tuberculosis peptidoglycan polymerization complex. We show that CrgA interacts strongly with FtsZ, FtsQ, CwsA and the class B penicillin binding proteins such as FtsI and PBPA in M. smegmatis. CwsA a binding partner of CrgA binds to Wag31 another key protein in peptidoglycan synthesis. Co-localization of CrgA with FtsZ in the mid cell region facilitates the Z-ring formation during the cell division process. Thus structural characterization of CrgA in a lipid bilayer is essential. Full length CrgA has 2 transmembrane (TM) helices, a 32 residue N terminal domain, a very short C terminal domain, and a 10 residue interhelical loop for a total of 93 residues and an 11.4 kDa molecular weight. Full length 15N, 13C uniform and amino acid specific labeled CrgA was over-expressed and purified from E. coli with a yield of 35 mg/L. To mimic the membrane environment, isotope labeled CrgA was reconstituted in POPC:-POPG liposomes. Orientation dependent 2D Polarization Inversion Spin Exchange at Magic Angle (PISEMA) and 3D Magic Angle Spinning (MAS) solid state NMR experiments were performed to obtain structural restraints. For all ssNMR experiments Low E probes in 600 and 900 MHz magnets were used at the NHMFL. CrgA TMD structure was calculated by CS-Rosetta and XPLOR-NIH. Structure refinement was enhanced by restrained molecular dynamics in a lipid bilayer environment.

**1125-Pos Board B17**

**Tertiary Structural Models for a Three Helix Membrane Protein in a Bilayer Environment from Oriented Sample Solid State NMR Data**

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1/3 of the world's population is infected with Mycobacterium tuberculosis 10% of whom will become sick from the bacilli. Multidrug resistant strains immune to the leading tuberculosis antibiotics, isoniazid and rifampicin, have emerged necessitating new treatments. Resuscitation from the dormant state is a key point at which infection can be fought. Cell wall reorganization is important for this activity and relies on transglycosylase enzymes to synthesize and degrade the peptidoglycan component. These enzymes can have membrane anchors and could therefore be regulated by membrane proteins. The gene product Rv1861 is identified by a domain signature associating it with transglycosylase proteins. The, short, 101 amino acid protein contains three transmembrane helices and the Walker A nucleotide binding motif. Structural characterization of membrane proteins of this size has been challenging using conventional methods. Solid state NMR is a technique that is well suited for studying these intermediate size membrane proteins. Most importantly it allows experiments to be performed in a native-like lipid bilayer environment.

Here we present a characterization of the secondary and tertiary structure of Rv1861 in a synthetic lipid bilayer environment. Using oriented sample solid state NMR we characterized the tilt and rotation of each helix in Rv1861. A set of tertiary structural models were created using the helix orientations and the typical structural contributions of certain amino acid types in the asymmetric membrane environment. These structural models allow for targeted measurements of structural restraints. Magic angle spinning solid state NMR of the protein in liposomes is ideally suited to measure distance that are essential for providing the correct tertiary fold of the transmembrane helices of Rv1861. Initial results in this direction will be presented.

**1126-Pos Board B18**

**Investigating the Interaction Between Hcf106 Peptides and the Phospholipid Bilayer by Solid-State NMR Spectroscopy**

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In plant cells most thylakoid lumen proteins are synthesized in the cytosol as higher molecular weight precursors containing targeting sequences, which are imported into chloroplasts and localized to thylakoid lumen for function. Two thylakoid transport systems direct precursors to the thylakoid lumen. Our lab focuses on one of those transport systems: cpTat (chloroplast Twin arginine translocation) system, which transports fully folded proteins utilizing the trans-thylakoidal proton motive force as its only energy source. The cpTat pathway consists of three membrane-bound subunits, Tha4, Hcf106, and cpTatC. cpTatC and Hcf106 form a receptor complex where the precursor binds to initiate the translocation events, whereas Tha4 is initially found as a separate complex that joins the receptor once the precursor has bound. Hcf106 is predicted to contain a N-terminal transmembrane domain (TMD), followed by an amphipathic helix (APH) and an unstructured C-terminus. However, detailed structural information of Hcf106 remains unknown. In this study, peptides encompassing the Hcf106 TMD or APH domain were incorporated into POPC and POPC/MGDG vesicles and analyzed by solid-state NMR spectroscopy. Using $^{31}$P NMR we show that Hcf106 TMD/APH interact with the phospholipid headgroups. $^{2}$H-NMR revealed the perturbation of the deuterated acyl chain by the peptides. Lastly we used CD spectroscopy to analyze the secondary structure of Hcf106 TMD and APH peptides. The experimental results suggest: (1) Hcf106 TMD and APH peptides interact with both POPC and POPC/MGDG lipids with the lipid composition influencing the peptide-lipid interaction. (2) Hcf106 TMD and APH form alpha helical structures to varying degrees under the same conditions. (3) Hcf106 APH appears to be parallel to the membrane surface with a slight tilt into the hydrophobic region of phospholipids, whereas the TMD peptide appears to be perpendicular to the membrane surface.

**1127-Pos Board B19**

**Characterization of Phospholamban Mutants Implicated in Dilated Cardiomyopathy**

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Contractility of heart muscle relies heavily on calcium ion messaging. Heart muscle relaxation takes place when this secondary messenger is transported against the concentration gradient from sarcoplasmic reticulum to myoplasm, performed by $Ca^{2+}$-ATPase (SERCA). The function of this calcium pump is inhibited by membrane protein phospholamban (PLN), a process that is reversed upon phosphorylation of PLN's cytoplasmic domain at S16 or T17. In the recent years several dysfunctional PLN analogs have been identified in patients with impaired heart contractility. Interestingly, the mutations were particularly prominent at R9 hotspot, where substitutions of an arginine residue to Cys, Leu or His have been identified.

We seek to deduce the molecular mechanism behind the interaction of these proteins, to gain the atomic insights into the regulation of contractility. For investigating the protein-protein and protein-membrane interactions, we employ an array of biophysical techniques, with an emphasis on NMR data. Conventional solution NMR techniques are used to probe the structure as well as fast/slow dynamics. Magic angle spinning solid-state NMR is applied for proteins with long correlation times to obtain the insights into the structure in the native lipid bilayer environment and the residues involved in protein-protein interaction. Oriented solid-state NMR gives information on the topology of the proteins with respect to the frame of the biomembrane.

The ultimate goal is the development of ways to manipulate calcium signaling *in vivo*. By investigating the interactions between the mutant proteins involved in pathologies, such as dilated cardiomyopathy, we intend to build a picture of the pathways at the molecular level. This will permit developing methods for regulating the proteins with abnormal functioning, allowing to control their function under pathological conditions.

**1128-Pos Board B20**

**Secondary Structure and Topology of the Transmembrane Domain of LspA, from Mycobacterium Tuberculosis, using Solid State NMR Spectroscopy: Initial Study**

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Lipoprotein Signal Peptidase A (LspA) belongs to type II signal peptidases and it is an essential enzyme in one of the three distinct steps in lipoprotein maturation. After targeting the prolipoprotein, LspA cleaves the signal peptide to result in the signal peptide cleaved lipoprotein, which is the precursor for the mature lipoprotein.

In Mycobacterium tuberculosis (Mtb), LspA is encoded by the gene Rv1539 resulting in a 202 amino acid residue peptide with a molecular weight of 21 kDa that is predicted to possess four transmembrane helices. It has been recognized in vivo that LspA is an essential protein for Mtb virulence and that it may also contribute to antibiotic resistance. As a result LspA has been identified as a potential drug target for the treatment of tuberculosis. Globomycin, a cyclic-peptide is known to inhibit LspA in a non-competitive manner.

Solid-state NMR enables studies of membrane proteins in a lipid bilayer environment. Since the native-like environment is important for studying the structure and function of membrane proteins, ssNMR is increasingly useful. In Oriented Sample ssNMR used here, the protein is uniformly aligned with respect to the magnetic field and 15N spectra of isotopically labeled protein have provided initial structural insights.

Here, all sample preparation steps and initial spectroscopy will be emphasized. Expression of LspA in E.coli, purification using IMAC, reconstitution of LspA into proteoliposomes via dialysis and the preparation of bicelles as an alignment media with different types of lipids and detergents will be presented. 1D NMR 31P and 15N spectra and 2D separated local field NMR spectra of uniformly and selectively labeled LspA will be shown.